β-Gal Reporter Gene Assay, chemiluminescent

Chemiluminescent assay for the quantitative determination of β-galactosidase activity in transfected cells

Cat. No. 11 758 241 001

500 Assays (microplate format)
250 Assays (tube format)

Version 07
Content version: July 2018

Store at +2 to +8°C

1. What this Product Does

Number of Tests

The kit contains reagents for performing 500 assays (microplate format) or 250 assays (tube format).

Kit contents

		Content	Color Code
1.	**β-Gal substrate (100×),** Galacton Plus	0.5 ml	red screw cap
2.	**Assay buffer**	50 ml	red screw cap
3.	**Enhancer**	8 ml	blue screw cap
4.	**Initiation solution**	40 ml	blue screw cap
5.	**Protease inhibitors**	5 tablets	white screw cap
6.	**Lysis buffer (5×)**	50 ml	black screw cap
7.	**Positive control,** β-galactosidase (*E. coli*)	0.5 mg	violet screw cap

Stability and storage

If stored at +2 to +8°C, the kit is stable until the expiration date printed on the label.

Assay time

90 – 150 min

Application

The β-Gal Reporter Gene Assay, chemiluminescent, is used to quantitatively measure β-Gal expression in eukaryotic cells that are transfected with a plasmid bearing the β-Gal-encoding lacZ reporter gene .

2. How to use this Product

Equipment and additionally required solutions

The β-Gal Reporter Gene Assay can be performed in all automated or manual luminometers in tube and microplate format, as well as in scintillation counters. When using the microplate format, black or white microplates must be used.

Except for redist. water (required for reconstitution and dilution purposes), the kit contains all assay reagents for lysing cells and determining β-galactosidase activity.

For standard cell washing steps, phosphate buffered saline (PBS) is also required.

2.1 Preparation of working solutions and stability

Substrate Reagent (solution 1)

Prepare substrate reagent freshly before use. Mix 1 volume β-Gal Substrate (bottle 1) with 99 volumes Assay Buffer (bottle 2).

Stability: The working solution is stable at least for 24 h at +2 to +8°C.

Initiation Reagent (solution 2)

Mix 1 volume of Enhancer (bottle 3) with 5 volumes of Initiation Solution (bottle 4) and mix thoroughly.

Stability: The Initiation Reagent is stable for at least 4 weeks when stored at +2 to +8°C.

Lysis Reagent, 1× (solution 3)

Prepare 1× lysis reagent by adding 10 ml of 5× lysis buffer (bottle 6) to 40 ml redist. water. Dissolve one protease inhibitor tablet (bottle 5) into the prepared solution.

Stability: 1× Lysis reagent without protease inhibitors is stable at −15 to −25°C or for 3 months at +2 to +8°C. Lysis reagent containing protease inhibitors is stable for one week when stored frozen at −15 to −25°C.

Positive Control (solution 4)

Reconstitute the content of bottle 7 with 0.5 ml redist. water. The solution contains 1 mg/ml β-galactosidase (*E. coli*) with a specific activity of approximately 600 Units/mg (as determined with ONPG as substrate).

Stability: The reconstituted enzyme solution is stable for 1 week at +2 to +8°C. When stored frozen in aliquots at −15 to −25°C the enzyme is stable for 3 months. Avoid multiple freezing and thawing.

2.2 Procedure

We recommend the following standard volumes for the β-Gal Reporter Gene Assay. Changing relative amounts and concentration may result in reduced sensitivity. If the recommended volumes are not practical (e.g. due to fixed instrument settings), all volumes should be adjusted in the same relation.

Assay component	Volume/test (working solution)	Total volume/kit (working solution)
Lysis reagent	0.25-1 ml per 6 cm culture dish or 1 ml per 10^6-10^7 suspension cells	250 ml
Cell lysate or positive control	50 µl/test	250 ml
Substrate reagent	100 µl/test	50 ml
Initiation reagent	50 µl/test	48 ml

Preparation of cell extracts and positive control

Preparing cellular extracts using the detergent lysis reagent offers the advantage that (i) very mild conditions are used, (ii) adherent cells do not need to be scraped from the culture dish, (iii) samples are processed very rapidly, (iv) thereby markedly facilitating β-Gal determination in large scale experiments. Another advantage of the lysis buffer is, that it is fully compatible with the Luciferase Reporter Gene Assay and ELISA procedures for the determination of CAT, or β-Gal levels using the non-isotopic CAT ELISA or β-Gal ELISA kits from Roche.

❶	Wash adherent cells three times with pre-cooled PBS (+2 to +8°C). Carefully remove PBS. Centrifuge suspension cells for 10 min at 250 × *g* and discard the culture medium. Wash cells twice with 5 ml of pre-cooled PBS (+2 to +8°C) and spin the cells again for 10 min at 250 × *g*. Carefully remove PBS.
❷	Add 1 ml of cell lysis reagent (solution 3) per 10^6 – 10^7 cells grown in suspension, or per 6 cm culture dish. Allow cells to stand for 30 min at +15 to +25 °C. [All soluble cytoplasmic and nucleoplasmic components, including β-Gal, will be extracted by the lysis buffer. In the case of adherent cells, nuclei (including DNA packed in chromatin) will remain attached to the vessel surface (3)]. Alternatively use 250 µl of cell lysis reagent per 6 cm culture dish and detach cells from the plate using a rubber-tipped scraping utensil (*e.g.*, rubber policeman).
❸	Transfer cell extract into a microfuge tube. Centrifuge for 2 min at maximum speed at +2 to +8°C to precipitate any cellular debris. Transfer supernatant to a clean microfuge tube. Cell extracts should be used immediately for the β-Gal determination or should be stored frozen at −70° C.
❹	For assay control or for internal standardization, dilute β-galactosidase positive control (solution 4) in lysis reagent (solution 3) by serial dilution steps. High dilutions of the β-galactosidase control can be stabilized by adding 1 mg/ml BSA.
❺	When measuring β-galactosidase activity in cells with a high endogenous β-galactosidase level, we recommend using non-transfected cells as control and/or to perform a heat inactivation step as described.

Protein determination

Results have to be normalized with respect to protein concentration or cell number.
For protein determination use copper-based protein assays *e.g.*, according to Lowry (4). Be aware that higher detergent concentrations may interfere with determination method. Therefore, check for interference or correct the calibration curve by addition of an equal amount of detergent lysis buffer. Protein determination should be performed in the linear range of the calibration curve. If absorbance in the sample is in the non-linear range, we recommend repeating the protein determination to obtain reliable results.
Volumes of samples should be adjusted so that the absorbance of the sample falls within the linear range. Do not dilute the cell extracts before performing the protein determination.

Alternatively different methods for determination of cell numbers can be used for normalization *e.g.,* measurement of metabolic activity by cleavage of the tetrazolium salt WST-1.*

How to use WST-1 Assay

❶	Perform cell culturing and transfection according to your standard protocol.
❷	30-150 min before cell lysis, add 10% WST-1 reagent to the cell medium.
❸	Quantify conversion of WST-1 directly from an aliquot, using an ELISA reader.
❹	Withdraw reagent/medium and lyse cells for reporter gene assay.
❺	Normalize reporter results according to the absorbance of the WST-1 assay.

Heat Inactivation (optional)

- Depending on the cell line or tissue under investigation, substantial amounts of endogenous lysosomal β-galactosidase may be present in the lysate and contribute to the non-specific background. This may not be a problem at high expression levels. However, when evaluating weak promoters, an increased background leads to reduced sensitivity. To reduce background produced by eukaryotic β-galactosidase incubate cell lysates for 60 min at 50°C (5).

Enzyme Reaction

All reagents should be fully equilibrated to room temperature (+15 to +25°C) before starting the test. Reagents with different lot numbers must not be used in one test series.

- Adjust the protein concentration in all cell extracts of one series to a value in the range of 0.1 – 1.5 mg/ml by dilution with lysis buffer. As a general rule, the protein concentration should be maintained as high as possible within this range. Protein does not interfere with the assay non-specifically.
- Pipette 50 µl of the cell extracts or positive control per well of the microplate (black or white) or per tube. Add 100 µl of the substrate reagent (solution 1) and cover the MP with a cover foil and tubes with Parafilm to avoid evaporation. Incubate for 15 min to 1 h at +15 to +25 °C, while gently rocking.

Initiation and measurement of the light reaction

- **Manual initiation (luminometer or β-scintillation counter):** Add 50 µl initation reagent (solution 2) per well/tube. Transfer MP/tubes to the luminometer or liquid scintillation counter. For best accuracy the addition of the initation solution should be timed in the same interval as the luminometer/LSC reads the samples. Integrate light production for 1 – 20 s (we recommend a 5 s integration time). The light emission peaks within 1 s and then decays with a half-life of about 10 min. Quantitative results can be obtained up to 30 min after addition of initation reagent. This feature might be especially advantageous when using a liquid scintillation counter for quantification (use the single photon monitor).
- **Automatic initiation (luminometer):** Transfer MP/tubes to the luminometer. Inject 50 µl initation reagent (solution 2) automatically. After a delay of 1 s, start integration of light production for 1 – 20 s (using an EG & G Berthold luminometer we recommend a 5 s integration time). If a higher initation volume must be chosen, dilute the initiation reagent with redist. water as indicated in the table (the amount of the initation reagent has to be one fourth of the final volume).

Cell Extract	Solution 1	Initation volume (= Initation reagent + x µl H_2O)	Final volume
50 µl	100 µl	50 µl (= 50 µl + 0 µl)	200 µl
50 µl	100 µl	100 µl (= 62.5 µl + 37.5 µl)	250 µl
50 µl	100 µl	150 µl (= 75 µl + 75 µl)	300 µl
50 µl	100 µl	200 µl (= 87.5 µl + 112.5 µl)	350 µl

Interpretation

- Plot the chemiluminescence signals obtained on the y-axis against the β-Gal standard concentrations on the x-axis to obtain a calibration curve.
- β-Gal concentration of unknown samples are obtained by plotting the observed signal on the y-axis, extrapolating to meet the calibration curve and reading the resulting β-galactosidase concentration from the x-axis. To obtain reliable results, the signal of the sample should be within the linear portion of the calibration curve.
- To allow direct, quantitative comparison of data obtained in independent experiments, a separate calibration curve must be established for each test series.

3. Additional Information

How this Product Works

Transcriptional activity of eukaryotic promoters is generally studied by linking an easily detectable reporter gene to the regulatory sequence of interest (1). The lacZ gene of *E. coli* encodes for β-galactosidase. When fused to eukaryotic promoters, it is one of the standard reporter genes. Bacterial β-galactosidase (β-Gal) consists of four identical subunits, each with a molecular weight of 116 kDa, which can be directly detected in extracts from transfected cells. To achieve this, the enzymatic activity is traditionally measured with the colorimetric substrates ONPG (2-nitrophenyl- D-galactopyranoside), or CPRG* (chlorophenolred-D-galactopyranoside).

With chemiluminescent substrates based on 1.2-dioxetanes, the sensitivity for enzymatic detection of β-Gal is increased by several orders of magnitude compared to the colorimetric substrates (2). By using this technology the β-Gal Reporter Gene Assay allows the investigation of weak promoters by a highly convenient non-isotopic method.

Test Principle

The outstanding sensitivity of this reporter gene assay results from the features of the dioxetane-class β-galactosidase substrate and the two-step assay principle (fig. 1):

Enzyme Reaction

In the first step the substrate, Galacton Plus, becomes deglycosylated by the enzymatic activity of the β-galactosidase contained in the sample. This in incubation step is performed at pH 7.8, where bacterial β-galactosidase is highly active. At this neutral pH, the cleaved dioxetane is protonated and will not produce a light signal. This stable intermediate accumulates during the reaction, and provides a light signal once the pH is adjusted higher.

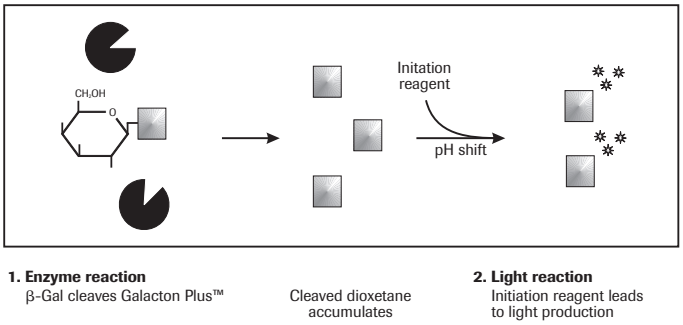

Fig. 1: Test principle

Light reaction

The light reaction is initiated by shifting the pH to a value higher than 12. Due to this shift in pH the activated intermediate becomes deprotonated and decomposes with the emission of light (475 nm). The presence of special polymeric enhancer substances improve the quantum yield of the chemiluminescent reaction. Under reporter gene assay conditions the light signal peaks within a second and then decays with a half-life ($t_{1/2}$) of approx. 10 min (fig. 2).

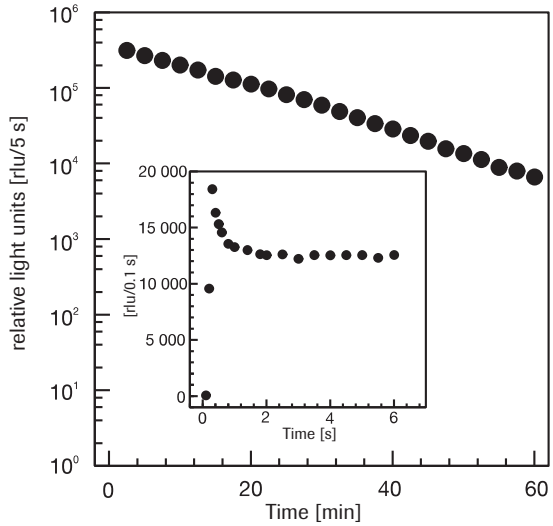

Fig. 2: Kinetic of light reaction under reporter gene assay conditions as described in section 2.2 Procedure (2 ng β-galactosidase).

Product characteristics

Detection Range and Sensitivity

Under assay conditions the detection range for the positive control is between 20 fg and 20 ng (fig. 3). However, the exact detection limit and measuring range depend on the measuring device and the measuring conditions used.

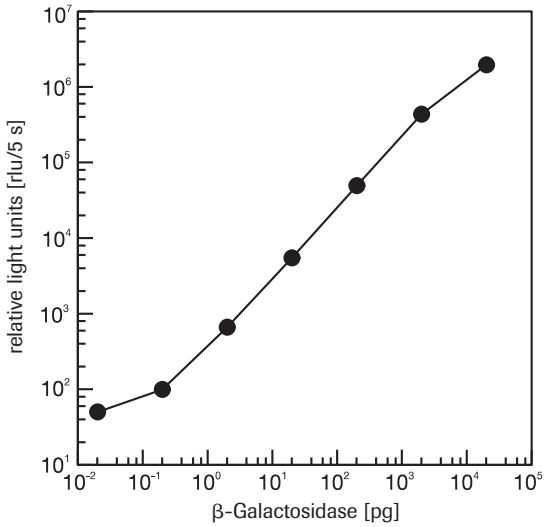

Fig. 3: β-Gal calibration curve. The determination was performed in a black microplate as described in section 2.2. The signal was integrated on a Berthold LB 96 P Luminometer for 5 s after a delay of 1 s.

Specificity

The β-Gal Reporter Gene Assay is designed for specifically measuring bacterial β-galactosidase activity. To achieve this, the enzyme reaction is conducted at a pH that optimizes for bacterial β-Gal, but allows no significant endogenous eukaryotic β-galactosidase activity. However, if very high endogenous β-galactosidase activity is found (*e.g.*, with hepatic cells), a heat treatment can be performed to ensure the specific determination of bacterial β-galactosidase encoded by the transfected plasmid.

References

1 Alman J. & Cook J.L. (1990) *Anal. Biochem.* **188,** 245.
2 Bronstein I. *et al*. (1994) *Anal. Biochem.* **219,** 169.
3 Lu, J. & Jiang, C. (1992) *BioTechniques* **12,** 643.
4 Lowry O. H. (1951) *J. Biol. Chem.* **193,** 265.
5 Young D.C. et al. (1993) *Anal. Biochem.* **215,** 24.

4. Supplementary Information

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Ordering Information

Product	Pack size	Cat. No.
Reporter Gene Assay Kits		
CAT ELISA	1 kit (192 tests)	11 363 727 001
β-Gal ELISA	1 kit (192 tests)	11 539 426 001
hGH ELISA	1 kit (192 tests)	11 585 878 001
Luciferase Reporter Gene Assay	1 kit (200 tests) 1 kit (1000 tests)	11 669 893 001 11 814 036 001

Product	Pack size	Cat. No.
SEAP Reporter Gene Assay	1 kit (500 assays MP format, 250 assays tube format	11 779 842 001
Detection and Quantification of reporter genes		
Aprotinin	10 mg 50 mg 100 mg	10 236 624 001 10 981 532 001 11 583 794 001
CPRG	250 mg	10 884 308 001
X-Gal	100 mg 250 mg 1 g 2.5 g	11 680 293 001 10 651 745 001 10 745 740 001 10 703 729 001
cOmplete	20 tablets in a glass vial (for 50 ml each) 3 x 20 tablets in glass vials (for 50 ml each).	11 697 498 001 11 836 145 001
cOmplete, Mini	25 tablets in a glass vial (for 10 ml each)	11 836 153 001
cOmplete, EDTA-free	20 tablets in a glass vial (for 50 ml each) 3 x 20 tablets in glass vials (for 50 ml each).	11 873 580 001 05 056 489 001
cOmplete, Mini, EDTA-free	25 tablets in a glass vial (for 10 ml each)	11 836 170 001
cOmplete	20 tablets in foil blisters, (for 50 ml each)	04 693 116 001
cOmplete, Mini	30 tablets in foil blisters, (for 10 ml each)	04 693 124 001
cOmplete, EDTA-free	20 tablets in foil blisters, (for 50 ml each)	04 693 132 001
cOmplete, Mini, EDTA-free	30 tablets in foil blisters, (for 10 ml each)	04 693 159 001
Transfection Reagent		
DOTAP Liposomal Transfection Reagent	5 × 0.4 mg	11 202 375 001
WST-1 Cell Proliferation Reagent	25 ml (2,500 tests) 8 ml (800 tests)	11 644 807 001 05 015 944 001
X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1.0 ml 5 × 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
X-tremeGENE HP DNA Transfection Reagent	0.4 ml 1.0 ml 5 × 1 ml	06 366 244 001 06 366 236 001 06 366 546 001

5. Short protocols

Required solutions

Solution	Content	Used for
1	**Substrate Reagent**: Prepare substrate reagent freshly before use. Mix 1 volume β-Gal Substrate (bottle 1) with 99 volumes Assay Buffer (bottle 2)	Step 3
2	**Initation Reagent:** Mix 1 volume of Enhancer (bottle 3) with 5 volumes of Initiation Solution (bottle 4) and mix thoroughly.	Step 4
3	**Lysis Reagent:** Prepare 1× lysis reagent by adding 10 ml of 5× lysis buffer (bottle 6) to 40 ml redist. water. Dissolve one protease inhibitor tablet (bottle 5) into the prepared solution	Step 1
4	**Positive Control**: Reconstitute the content of bottle 7 with 0.5 ml redist. water (final conc.: 1 mg/ml)	Preparation of β-Gal calibration curve

Working procedure flow chart: Basic protocol

Step	Procedure	Reagent/Volume	Time/ Settings	Temp.
	Pre-equilibrate all reagents and samples fully to +15 to + 25°C			+15 to +25°C
	Wash cells	PBS	2 times	+2 to +8°C
1	Cell lysis	1 ml lysis reagent (solution 3) per 10^6 – 10^7 suspension cells or a 6 cm culture dish	30 min	+15 to +25°C
2[1)]	Heat inactivation (optional)	cell lysate	60 min	+50°C
3	Enzyme reaction	50 µl sample + 100 µl substrate reagent (solution 1)	15 – 60 min	+15 to +25°C
4	Light reaction	50 µl initiation reagent (solution 2)	measurement after 1 s to 15 min	+15 to +25°C
5	Signal integration	—	1 s delay, integration for 5 s	—

[1)] The heat inactivation step is only required when extracts of cells with high endogenous β-Gal levels such as cells of hepatic origin are used.

Changes to Previous Version

- Editorial changes.

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

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